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Review Article

A SYSTEMIC REVIEW ON TUBERCULOSIS: DIAGNOSIS, TREATMENT AND CHALLENGES

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ABSTRACT

In developed countries worldwide, tuberculosis is an endemic condition. The early and conclusive diagnosis of Active Pulmonary Tuberculosis (TB) is important for avoiding death and spreading disease, both at the person and population level. Diagnostics commonly available can be widely divided into techniques focused on microscopy, culture, and molecular methods. The Nucleic Acid Amplification Test (NAA), Line Probe Assay, and Cartridge Based Nucleic Acid Amplification Test (CB NAAT) are three advanced molecular approaches that are listed. All these diagnostic strategies, however, either have weakened sensitivity or require costly instruments that eventually restrict their usefulness as a proven form of diagnosis. Sputum microscopy, which detects Mycobacterium tuberculosis (Mtb) in a clinical sample, is currently used as an easy and inexpensive diagnostic test for TB. The sub-optimal sensitivity of this test is based first on the abundance of Mtb in the sample and secondly on the accuracy of the operator. Culture-based diagnostic studies, on the other hand, are time intensive and reliant on knowledge and infrastructure. Likewise, molecular experiments often rely on expensive instrumentation and complexity in scientific visualization. An easy, sensitive and inexpensive diagnostic approach for early-stage Active Pulmonary Tuberculosis diagnosis is therefore still a primary requirement.

Keywords: Tuberculosis, Diagnosis, Microscopy, Culture, NAA, Line Probe Assay, CBNAAT.

1. INTRODUCTION

Active Pulmonary Tuberculosis (Active TB) is a pathological condition caused by the chronic infection of *Mycobacterium tuberculosis* (Mtb). The infection spreads through air and primarily infects lungs. As per recent worldwide disease monitoring, the country with highest instances of this disease is Swaziland, southern Africa (With 1200 cases per 100,000 persons reported, in the year 2007) [1]. In developed countries like United Kingdom, this is an urban disease whereas in Western Europe, 0.3 % of the population is TB affected [2, 3]. In India largest incidence of TB was detected in 2014 when 2.2 million new cases were traced with 0.3 million death [4, 5]. Even in current dates, two deaths of Active TB patients occur in every three minutes within India. In rural areas of many States, lack of sufficient health care infrastructure is a significant impediment to controlling and eliminating this fatal disease [6, 7]. In cooperation with the private sector and the Indian Medical Association, the National Tobacco Program (NTCP) has taken many steps to address the difficulties of treating this disorder.

The primary step for the management of any pathological condition is the proper and definitive diagnosis of the same in an endemic population [8]. Several screening procedures are required for tuberculosis to diagnose both latent and active infections. Latent infection is detected through Tuberculin skin test and Interferon gamma release blood test for long time although these tests do not guarantee accurate results. Chest X-ray was used as a method of diagnosis of the TB infection in earlier days, however the method is not specific. Sputum Smear microscopy and its adaptations, such as fluorescent microscopy, LED microscopy, and Ziehl-Neelsen light microscopy, have been used to detect BMD in sputum patient samples. These researches show active tuberculosis infection. To detect patient antibodies against a Mtb virus, a serological screening, ALS (Antibodies from Lymphocyte Secrestion), and Transdermal Patch Injection Test are used. Antibody development is dependent on the immune response of particular patients, and antibodies are only in circulation for a limited period, so these trials are insufficiently receptive. The World Health

Organization (WHO) approved the most recent Effective TB diagnostic assay in 2010, which used Nucleic Acid Amplification (NAA) for definitive Mtb genome diagnosis. [9]. In addition, based on a mutation in the Mtb genome, this trial can diagnose multi-medicine resistant (MDR) tuberculosis infection. Since widely imported test cartridges are costly, a discounted price is imposed in TB-endemic countries. The aim of this research is to look at all of the existing methods of tuberculosis diagnostic tests, as well as the benefits and drawbacks of each. The paper would also provide a potential outlook on the present situation, which will aid in the development of recommendations for a tuberculosis diagnosis that is simpler, more definitive, less expensive, and more adaptable [10].

2. CLINICAL MANIFESTATIONS OF TUBERCULOSIS

The clinical manifestations of any disease are the first tools to diagnose any illness. Similarly for tuberculosis, there are several clinical manifestations which prove to be the first signs for identification of TB. Nevertheless, many misleading as well as non-specific symptoms in the cases of tuberculosis infected patients especially in children and elderly, make the diagnosis very tough and complex. Additionally, challenge to the timely detection in cases of extra-pulmonary tuberculosis has been faced in several cases. The clinical manifestations for the diagnosis of TB in children, elderly as well as for extra-pulmonary TB has been discussed below in details.

2.1. Tuberculosis In Children

The paucibacillary nature of tuberculosis in children, poses a difficulty in its diagnosis. In major reported cases in the developed and developing countries the bacteriological confirmation [11, 12] is less than 30-40%. As a result, the diagnosis in children suffering from tuberculosis in a setting where scarcity of testing resources or tools exists largely is dependent of the clinical manifestations, history of contact with patient suffering from tuberculosis, and specific tests like TST and chest radiography is accessible. Maries et al. devised an approach combining a continuous non-remitting cough that lasted for more than 2 weeks, fatigue and well documented deterioration of health since the previous 3 months or more. These symptoms provided a reasonable along with reliable precision in the diagnosis of tuberculosis in children uninfected with HIV. However, the detection accuracy was found to be lower in HIV infected children as compared to low risk group. This again led to a serious setback in the accurate diagnosis of tuberculosis in children at low resource setting and high HIV score [13].

2.2. Tuberculosis in old age

Tuberculosis has also been known to affect geriatric population predominantly, especially in low prevalence situation. The geriatric subjects in which the immunity has been compromised due to age or other clinical comorbidities, are at higher risk to suffer from tuberculosis. Due to the absence or compromise of immunity, tuberculosis presents 'atypical' clinical manifestations in older subjects [14, 15]. Extra-pulmonary tuberculosis, comprising of military disease are more frequently observed in older patients than pulmonary tuberculosis [16]. Haemoptysis, sweating and fever are less commonly observed in older patients, whereas, dysponea is a more regular symptom. Interestingly, the level of total serum protein, white blood cells, TST positive frequency along with the formation of cavity were reported to be much lower in old subject. However, similarity in the occurrence of the lesions in the upper respiratory tract and lungs were found in both age groups [17]. Yet another striking observation was made in the common chest X-ray test of the old or immune compromised patients suffering from tuberculosis, which revealed the presence of lesions in the lower part, accompanied by either basal thickening or effusion or both [18].

These atypical clinical manifestations accompanied by underlying illness add on to the complexities in the diagnosis of tuberculosis in elderly as well as in immune-compromised patients and often hamper the timely detection of the disease.

2.3. Delays in diagnosis

The uncertainty in the diagnosis of tuberculosis, which may be due to the health care system, i.e. the delay in the diagnosis of tuberculosis on the part of the hospital, or the delay on the part of the doctor, is also considered to be among the key measures that determine the consistency of the diagnosis. In this situation, the delay in the diagnosis can be explained as the time lapse from the first time the patient sees the doctor to determine the true diagnosis of tuberculosis.

Table 1: Treatment and differential diagnostic clinical presentations and laboratory tests for major types of extra-pulmonary tuberculosis [19, 20]

Site	Signs and symptoms	Laboratory test	Differential diagnosis
Pleurisy	Generally, primary cases of infection have an acute course of signs relative to reactivation-type TB. (non-productive) cough and chest pain (pleuritic, sharp, stabbing, breathing-related). For more advanced cases, feverishness, dyspnea, chills, sweat, weight loss. Generally exudates are TB pleural effusions	Radiography, sputum bacteriology (for undiagnosed pulmonary TB), pleural fluid thoracocentesis for cell profile, protein, pH, glucose, LDH, smear, culture, NAAT ADA,[21-23] IFN-g,[24] IGRA, [25-27] histology, culture and NAAT pleural biopsy	Effusion due to congestive heart failure, carcinoma, other rheumatological and infectious diseases.
Lymphadenopathy	Rare in the axillary & inguinal region, most commonly in the neck and head region. Right predominates, but multiple lesions have 1/4 biliateral & 78 percent. 41 percent are suffering from pulmonary TB. The lesion begins as a painless enlargement of the superficial LN, with no inflammation of the skin, and can then undergo pustulation and fistulation for several weeks or months. General signs are unusual in cases with limited lesions.	MTB, chest radiography, biopsy (total excisional) culture isolation accompanied by bacteriological culture/PCR and histology. Fine aspiration needle	Infections of NTM, lymphoma, sarcoidosis, Kikuchi disease, Castleman disease, Kimura disease, lymphadenitis corynebacterium pseudotuberculosis.
Bone & Joint	The spinal column, followed by the hip and knee, is the most common. Fever and wasting can occur in large inflammatory arrays, but the local manifestation predominates. The most renowned pain. Accumulation of soft tissue at or near the bone or joint focus (cold abscess). Neurological signs (weakness or numbness triggered by compression of the spinal cord)	Aspirate (abscess, synovial fluid) MTB and biopsy (e.g. synovia) specimens, CT & MRI.	Pyogenic, rheumatoid, gout, area osteoporpsis, idiopathic chondrolysis: for arthritis. For cystic lesions of the bone: eosinophilic granuloma, sarcoidosis, cystic angiomatosis, fungal infection, plasma cell myeloma, metastatic malignancy
Disseminated or miliary TB	The variation depends on the organs involved. Feverishness, fatigue or weakness, anorexia, lack of weight, headache (meningeal complication), pain/swelling in the abdomen (peritoneal involvement), cough.	Chest radiography, CT (HRCT), fiberoptic bronchoscopy, TBLB, haematology (often no abnormality in the beginning) (anaemia, leukopenia or leukocytosis, rarely leukemoid reaction). Liver function, biopsy of the bone marrow, biopsy of the liver (NAAT included), fundoscopy.	Alveolar microlithiasis, propagated carcinoma, NTM infections, sarcoidosis, pneumonitis with hypersensitivity.
Central nervous system	The crucial aspect of the procedure is the scale and position of the tuberculoma and the strain it produces. Early signs (feverishness, malaise, anorexia, irritability, headache) are compounded by	MTB (microscopy, culture and PCR), immunology (ELISA, IgG immune complex, antibody assays and IGRA), and ADA. Cerebrospinal fluid for pressure, cellularity, protein,	Some infections (fungal, infectious, trypanosomal, bacterial), vascular (emboli numerous, SBE, sagittal vein thrombosis), vascular collagen (SLE,

	neurological symptoms (progressive headache, lethargy, mood changes, lack of memory, reduced cognition, confusion), preceded by stupor-coma with or without neurological impairment.	glucose, Meningeal Biopsy Radiography, CT, MRI (histology, MTB).	polyarteritis, and others).
Abdominal	Frequency: peritonitis, accompanied by inflammation of ileocaecal, anorectal and mesenteric lymph nodes. Abdominal swelling, fever, ascites, pain, anorexia/weight loss are normal in peritoneal TB.62,63	Peritonitis: ultrasound, (M) laparoscopy (guided biopsy), culture paracentesis of ascites, and IGRA and ADA	Malignant ascites, incidental bacterial peritonitis cirrhosis, starch peritonitis, NTM peritonitis, sarcoidosis.
Pericarditis	Dyspnoea, tachycardia, distension of the neck vein, oedema, hepatomegaly, pulse paradox, pericardial rub, fever.74,75	Bacteriology, histology, IGRA,[28] and ADA pericardial tissue/fluid.80-82 Echocardiography [29], CT and MRI (pericardial effusion and thickening), (ECG (low voltage, T inversion) [30]	Infections of bacteria (e.g. Pneumococcus), viruses (e.g. CMV, HSV, Coxsackievirus) or fungi (e.g. Aspergillus); vascular disorders of collagen; uremia; post-myocardial infarction or post-pericardiotomy; malignancies; trauma.
Genitourinary	Dysuria, frequency, nocturia, urgency, back, flank or abdominal pain, testicular or epididymis tenderness/swelling, haematuria. Superimposed urinary tract inflammation in urinary stasis cases with other bacteria.88-93	MTB (smear, culture, PCR) urine or secretion (early morning sample), T ultrasound [31] simple abdominal radiograph, i.v. High-dose urography, image-intensified endoscopy, percutaneous antegrade pyelography, biopsy [32, 33] for possible genital damage. [34, 35].	Cystic kidney, pyelonephritis, xanthogranulomatous pyelonephritis, urinary malacoplacia, benign and malignant tumours.

Abbrevations- ada, adenosine deaminase; ct, computed tomography; ecg, electrocardiogram; hrct, high resolution computed tomography; igra, interferon-gamma release assay; ldh, lactate dehydrogenase; ln, lymph node; mri, magnetic resonance imaging; mtb, mycobacterium tuberculosis; ntm, non-tuberculous mycobacterium; pcr, polymerase chain reaction; sbe, subacute bacterial endocarditis; sle, systemic lupus erythematosus; tb, tuberculosis; tblb, transbronchial lung biopsy.

3. AVAILABLE DIAGNOSTICS FOR TUBERCULOSIS

Currently available screening methods for tuberculosis recognise the presence of Mycobacterium tuberculosis in patient samples. Tests vary only in bacterial detection mode and/or specificity, sensitivity, etc. The following section would describe the various methods used to diagnosis tuberculosis in depth.

3.1. Tuberculin skin test

For the diagnosis of the bacteria mycobacterium tuberculosis inpatient sample [36], the Tuberculin skin test or Montoux tuberculin test is used. Under the top layer of the skin on the inner forearm, tuberculin protein or a purified protein derivative is added. If the patient is infected with TB, the tuberculin antigen interacts with it and after two days a red bump appears on the site and the high or low risk is diagnosed by measuring the red bump size. The test above cannot discriminate between active or latent infections of TB. The BCG (Bacillus Calmette Guerin) vaccine is not a defense against TB infection as per US guideline, and a positive tuberculin test in patient receiving the BCG vaccine is viewed as latent TB infection. False-positive findings can also be obtained through hypersensitivity, allergic reactions, etc. Conversely, the UK does not recommend tuberculin skin testing in BCG vaccinated individuals. As a result, US tackles numerous incorrect diagnosis of latent TB infection, and the UK does not diagnose various latent TB infections [37]. This test for latent TB infection screening is not effective. In people with HIV infection or organ transplantation who have

been in close contact with an active TB patient, minimal responses (5 mm of firm swelling at the site) are considered to be positive. Higher reactions (greater than or equal to 10 mm) are reported in diabetes patients, kidney failure, or other conditions that increase their risk of developing active TB, as well as health care workers, drug users, immigrants who have recently arrived from a country with a high TB incidence, children under the age of four or teenagers exposed to high-risk adults, students, and others [38].

3.2. Interferon gamma release blood test

This test is mainly used for the diagnosis of latent TB infection and it generally releases specific interferon gamma to react with different antigens. Two types of test, Quantiferon Gold Test and TB Spot test is available under this test head [39].

The Quanti FERON-TB Gold test (QFT-G) is a whole-blood test for use as diagnosis of Mycobacterium tuberculosis infection [40] The US Food and Drug Administration (FDA) approved this test in 2005. Antigens and controls are used to combine blood samples. For QFT-G, the antigens contain synthetic peptide mixtures containing two M. Proteins for Tuberculosis, ESAT-6 and CFP-10. The sum of interferon-gamma (IFN-gamma) is measured after incubation of the blood with antigens for 16 to 24 hours to check whether the patient is contaimnated with the *M. tuberculosis* infection or not? In reaction to interaction with TB antigens, their white blood cells will release IFN-gamma. The findings of the QFT-G are based on the quantity of IFN-gamma produced in reaction to the antigens. To distinguish between a diagnosis of latent TB or active TB, clinical assessment and additional testing are needed. Advantages of the latter test provide reduced patient reporting time and less time to assess the results. Prior vaccination of BCG (bacilli Calmette-Guérin) does not affect this test [41].

The T-SPOT BLOOD TEST counts the number of anti-mycobacterial effector T cells, white interferon-gamma-containing blood cells, in a blood sample. This gives an overall evaluation of the host immune response to mycobacteria and may indicate the presence of contamination with Mycobacterium tuberculosis, the causative agent of tuberculosis (TB). Since this does not rely on the production of a healthy antibody response or a pathogen that can be retrieved, the technique can be used to detect latent tuberculosis [42]. The BCG vaccine does not impair this test and offers fast results. For arousal, all of these experiments use multiple antigens. Centered on the rules of the test, the T-SPOT test is thought to be more specific than the skin test for tuberculin.

3.3. Chest X-Ray

The standard view is a posterior-anterior (PA) chest X-ray; CT scans may be needed in active pulmonary TB, where infiltrates of the cavities are frequently seen in the upper lungs with or without mediastinal or hilar lymphadenopathy [18]. Lesions can, however, develop anywhere in the lungs. Any abnormality on a chest X-ray could indicate tuberculosis (TB) in HIV and other immunosuppressed individuals, or it could be completely normal. In the hill region or upper lobes, old healed tuberculosis typically occurs as pulmonary nodules, with or without fibrotic scars and volume loss [19]. Bronchitis and pleural scarring are possibilities. Nodules and fibrotic wounds contain slow-multiplying tubercle bacilli with the potential to produce active tuberculosis. Individuals who have these findings, regardless of age or whether they have a positive tuberculin skin test reaction, should be considered high-risk candidates for latent infection care. Calcified nodular lesions (calcified granuloma) on the other hand, have a very low chance of becoming active tuberculosis in the future. Chest radiograph abnormalities may be indicative of tuberculosis, but they are never conclusive [19]. Chest radiographs, on the other hand, can be used to rule out pulmonary tuberculosis in people who have a positive tuberculin skin test but no signs of the disease.

3.4. Sputum smear test

The first technique for TB to be used in countries where there is a high level of TB infection rate is also sputum smear microscopy. In the lungs and airways that connect to the lungs, sputum is a thick substance that is created. The person coughing usually takes a sample of sputum and generally recovers several samples of sputum [43].

It was suggested in 2012 that, without any loss of accuracy, two specimens could be collected on the same day [44]. To perform the TB test, a very thin layer of the substance is placed on a glass slide and referred to as a smear. The sample is then stained with a series of special stains, and the stained slide is examined under a microscope for evidence of tuberculosis bacteria [45]. Sputum smear microscopy is a simple and inexpensive procedure that can be learned quickly and easily. In addition, the findings are available within hours. The sensitivity, however, is only around 50-60%. The

diagnosis rate could be much lower in countries with a high incidence of both pulmonary TB and HIV infection, since many individuals co-infected with HIV and TB have very low levels of TB bacteria in their sputum and are thus identified as sputum-negative. A table on the specifications of collected specimens is given below:

Table 2: Biological specimen for the diagnosis of tuberculosis [45]

Specimen	Amount	Application	Preservation or Transport	Remarks
Sputum	2-5 ml	1, 2, 3	Unprocessed	3x in the morning on an empty stomach
Induced Sputum	2-5 ml	1, 2, 3	Unprocessed	Expectoration following inhalation of 3% NaCl solution
Bronchial secretion/ bronchoalveolar lavage	2-5 ml	1, 2, 3, 4	Unprocessed	On the day of sample selection, BAL-ELISPOT should be performed.
Gastric aspirate	>2 ml	1, 2, 3	In 1-2 mL phosphate buffer (trinatrium phosphate)	Only if sputum isn't available and bronchoscopy (BAL) isn't needed.
Biopsy, survival specimen	2 distinct portions (1) and (2)	1, 2, 3, 5	(1) In 0,9% NaCl for microbiological examination; (2) in formalin for histopathological examination	(1) Not in formalin
Pleural effusion, ascites	20 ml	1, 2, 3, 4	Unprocesssed	ELISPOT should be performed on the day of sample collection
Cerebrospinal fluid	2-3 ml	1, 2, 3, 4	Unprocessed	ELISPOT should be performed on the day of sample collection
Urine	30 ml	1, 2, 3	Unprocessed	-3x - First specimen of urine in the morning-Fluid restriction the evening/night before
Stool	5-10 ml	1, 2, 3	Unprocessed	-3x
Blood	5-10 ml	1, 2, 3, 4	Heparin- or lithium-citrate Tubes	- Indicated only in immunosuppressed patients - Do not use EDTA blood
Bone marrow	2 distinct portions (1) and (2)	1, 2, 3, 5	(1) In heparin- or lithium citrate tubes; (2) air-dried smears and/or formalin preserved biopsies	- Indicated only in Immunosuppressed patients - Biopsy or aspirate for (1) not in EDTA or formalin

Application in different tests: 1, microscopy; 2, culture; 3, NAAT; 4, IGRA; 5, histopathology. BAL, bronchoalveolar lavage; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; EDTA, ethylenediaminetetraacetic acid.

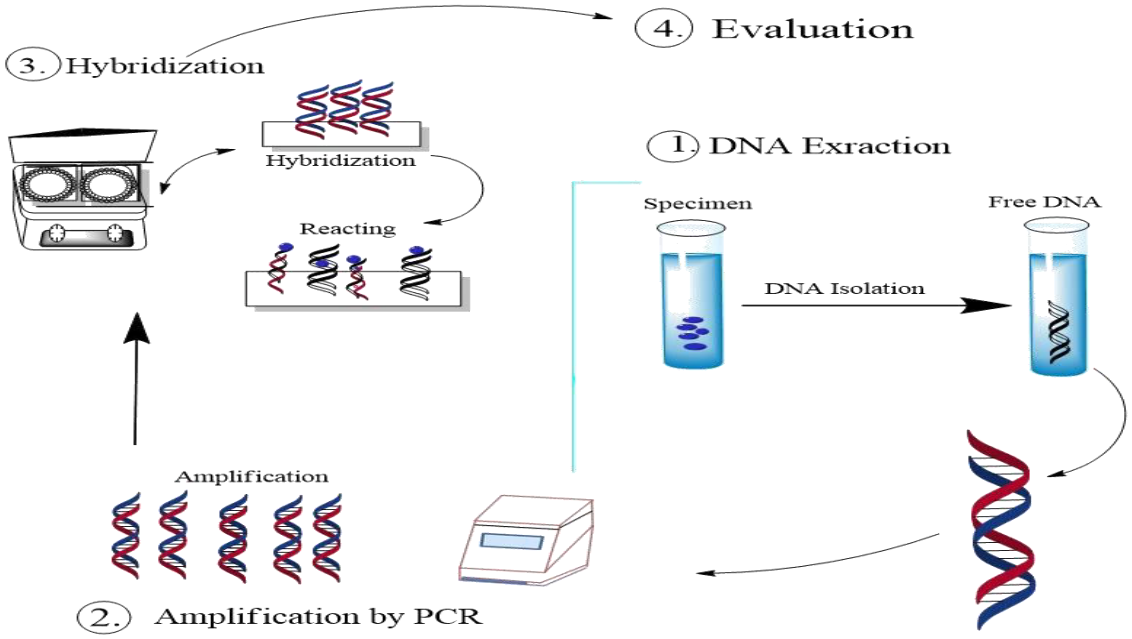
3.5. ALS test

The Lymphocyte Secretion Antibodies (ALS) assay is an immunological test that can be used to identify active diseases like tuberculosis, cholera, and typhoid. The ALS assay has recently gained popularity in the scientific community due to its ease of use for tuberculosis diagnosis. Rather than latent TB infection, the hypothesis is based on the release of antibodies from in vivo activated plasma B cells present in the bloodstream for a short period of time during active TB infection in response to TB antigens. PBMCs were isolated from blood on Ficoll-Paque and suspended in the culture medium of a 24-well tissue culture plate using differential centrifugation. At 37°C with 5 percent CO_2, different dilutions of PBMCs were incubated. Supernatants were obtained at 24, 48, 72 and 96 h after incubation and ELISA tested the supernatants against BCG or PPD. The positive or negative outcome is shown by the ELISA titre [46]. The diagnosis of TB is most complicated. Sputum culture is the basis of routine diagnosis for TB patients. Nevertheless, society takes 6-8 weeks of false positive outcomes of 10-20 percent [47]. It would be useful for physicians to perform a fast serological analysis for diagnosis, follow-up of disease

progression and response to treatment [26-47]. The Mantoux test is an important tool in the developed world for the diagnosis of latent TB infection and disease, but it has low predictive utility in people vaccinated with Calmette-Gue'rin (BCG) bacilli, as well as in people living in areas where TB is endemic. The purified protein derivative (PPD) skin test the poor predictive potential is due to cross-reactivity as well as false negative responses in malnourished children with BCG and atypical mycobacteria [48, 49]. In in vitro experiments, BCG was used as an antigen in EIAs to assess disease incidence, but its use was suspended due to difficulties in understanding, differentiating issues between active and past diseases, and poor sensitivity and specificity [50]. The lymphocyte secretion antibodies (ALS) assay was used earlier to detect a particular antibody reaction in healthy adults after oral vaccination with a killed cholera vaccine without any need for in vitro stimulation of the antigen [51]. A high sensitivity of >93 percent [34] is the key benefit of this system. This procedure does not include a sample taken from the site of the illness, and may also be useful for the diagnosis of childhood paucibacillary tuberculosis. Secreted antibodies for further study will be stored for a long time.

3.6. Nucleic acid amplification test

The Food and Drug Administration (FDA) approved a reformulated Amplified Mycobacterium Tuberculosis Direct Test* (MTD) (Gen-Probe®, San Diego, California) for detecting Mycobacterium tuberculosis in tuberculosis-positive and tuberculosis-negative respiratory specimens from AFB patients suspected of TB on September 30, 1999. (TB). The Amplicor® Mycobacterium Tuberculosis Test (Amplicor) (Roche® Diagnostic Systems, Inc., Branchburg, New Jersey) and another nucleic acid amplification test (NAA) were previously approved for direct identification of M. Respiratory specimens that have positive AFB smears have tuberculosis. This notice updates the initial 1996 summary [52] and includes recommendations for the use and analysis of NAA test findings for the treatment of patients suspected of suffering from TB. The number of specimens to be tested with NAA varies depending on the clinical condition, prevalence of tuberculosis (TB), prevalence of nontuberculous mycobacteria (NTM), and laboratory competence [53, 54]. The accompanying figure, based on available information, is a fair approach to NAA testing of respiratory specimens from patients with signs or symptoms of active pulmonary TB for whom a presumed diagnosis has not been identified.



On three different days, collect sputum specimens for AFB smear and mycobacterial culture. On the first collected sputum specimen, the first positive smear sputum specimen, and subsequent sputum specimens, perform the NAA test as shown below. If the first sputum sample is smear-positive and NAA-positive, the patient is likely to have TB without further NAA checks. However, because there is doubt about the existence of

NTM, nothing is applied to the diagnostic work-up by the NAA test. A screen for metabolites should be performed when the first sputum is smear-positive and NAA-negative. With Amplicor, the inhibitor test may be performed as an alternative. To search for MTD inhibitors, spike an aliquot with lysed M from the lysed sputum sample. Tuberculosis (approximately 10 or an equal quantity of M. tuberculosis rRNA per reaction) and repeat the test beginning with amplification.

Additional specimens (not to exceed three) should be checked if inhibitors are not found. If a second sputum specimen is smear-positive, NAA-negative, and has no inhibitors, the patient is suspected of having NTM. The NAA test is of no diagnostic benefit if inhibitors are found. Additional specimens should be screened with NAA (not to exceed a limit of three). If the sputum is smear-negative and MTD-positive, additional specimens can be tested with MTD (not to exceed three). The patient may be believed to have TB if a subsequent specimen is MTD-positive. An extra sample can be tested with MTD if sputum is smear-negative and MTD-negative. If both smear and MTD outcomes are unfavourable, the patient should be believed to be contagious. Since unfavourable NAA results do not rule out the possibility of active pulmonary TB, the clinician must rely on clinical judgement in making decisions about the need for antituberculous therapy and further medical work-up. If the replicate NAA test fails to confirm initial NAA test results, the clinician must rely on patient assessment when deciding if antituberculous therapy is needed. As a result, the patient's response to medication and cultural outcomes are used to confirm or refute a TB diagnosis. NAA testing can help confirm a diagnosis, but it can't replace an AFB smear or a mycobacterial culture, and it can't replace clinical judgement. These tests should be recognised by physicians based on the particular diagnosis, and NAA testing should be performed by laboratories only at the physician's request and on selected specimens. If this sacrifices the opportunity to conduct the other existing tests which have better-defined diagnostic usefulness and effects, laboratorians should not reserve material from clinical specimens for NAA testing. Due to unrecognised methodological variations and differences in cross-contamination rates, the accuracy of NAA tests varies between labs [55]. To minimise the risk of methodological mistakes, several specimens from the same patient may not be examined together. On all smear-positive and smear-negative respiratory specimens, laboratory directors can provide clinicians with details on the success of NAA tests in the local environment, including sensitivity and accuracy relative to community. Substantial inconsistencies with either culture or NAA technique can imply issues. It is therefore necessary to record the number of NAA experiments replicated due to the lack of negative and positive controls. Clinicians should consider the effect on the predictive value of the NAA test of improvements in susceptibility, precision, prevalence of TB, and prevalence of other mycobacterial diseases. No respiratory specimens or specimens from treated patients have limited knowledge about the accuracy of the NAA procedure. After cultures turn negative during therapy, NAA tests often remain positive and will remain positive even after therapy completion.

3.7. The line probe assay

The line probe assay can be a quick diagnostic tool that has aimed to locate active m. TB complex and rifampicin resistance alone or combined with INH. In this test, there is no specific marker, the aided diagnosis of Mycobacterium tuberculosis is phlegm in an overly patient. The line probe assay technique is doled out in the following steps, Positive culture DNA extraction or directly from biological specimens. Amplification of a particular gene region by using the polymer chain reaction method, then reverse coupling of the amplified portion to a specific DNA probe immobilized on the show bands of strip output. For a positive smear example, the line test measure shows high explicitness (>99 percent) and affectability (>97 percent) for rifampicin tolerance, along with specificity of INH (99 percent) and (90 percent) affectability.

3.8. CBNAAT

In non-Asian regions, the CBNAAT analysis is also known as Genexpert. In 2004, the test was released to the industry. Furthermore, the development of the test was done in 2008. In 2009, the first clinical trial was carried out. Genexpert is a hereditary examination diagnosing the use of T.B. The World Health Organization recommended that Genexpert be integrated into their health management system in the Gregorian calendar month 2010. Manipulation of TB microorganisms by Sputum Assay by sleuthing is a gerenral way which includes amplification steps that are expedient and easy to use and examine. It includes amplification steps that are expedient and easy to use and examine. It is expected to take less than two hours. Furthermore, CBNAAT recognizes the mole-cular

transformations associated with polymerase chain reaction obstruction of rifampicin drug. Genexpert is also licensed for irresistible and infectious diseases associated with MDR-TB or HIV according to WHO guidelines.

3.9. Fluorescence-activated cell sorting

Previous experiments have used antigen-stimulated cell immunophenotyping to achieve a faster diagnosis of TB than conventional methods, particularly in offenders whose AFB sputum smears have previously tested negative, with the aid of fluorescence-activated cell sorting of sputum cells [56] and BAL cells [57-59]. While study of multicolor flow cytometry makes and promotes the identification of cell populations reacting after an antigen association, compared to IGRA TB immunodiagnosis, this technique is said to be more complicated as well as challenging.

For lymphocytes unique to PPD, individuals with BAL cells, Sputum T cells and LBT1 cells [60] are particularly established. In addition, flow cytometry assays in which PPD functions as stimulants have been found to struggle to recognize individuals suffering from LBT1 and active tuberculosis.

Even so, it is difficult to use flow cytrometry as a very common method for the diagnosis of tuberculosis because of the low incidence of area of variation T cells unique to M.tuberculosis in the sputum, which serves as a stimulant for certain immune-based assays as well as flow cytometry cultures [61, 62]. Nevertheless, wherever appropriate, flow cytometry will serve as a very dependable as well as promising method to enhance the diagnostic accuracy for the diagnosis of AFB smear negative tuberculosis [63].

4. FUTURE PERSPECTIVE

To address the challenges of complete TB eradication, a more effective and efficient therapeutic approach is needed. Understanding the structure of bacteria, various aspects of their survival, biosynthesis of important components, and metabolism, and then developing novel therapeutic agents that target these crucial aspects of bacteria will help, or will not help, bacteria die. Specific recognition is greatly aided by the availability of mycobacterium genome sequences and genetic tools such as transposon mutagenesis, gene transfer, and knockout genes. Besides, targets should also be considered for drug development involving the biosynthesis of essential components of the bacterial cell wall as well as the pathogenesis of the disease. The use of multiple drug formulations that reach multiple targets in various pathways involves a system biology approach to achieve the desired effect, in addition to finding new targets.

Condition or disease (TB specification)	Drug	Phase	Primary outcome	Sponsors	Age group of the patients	Reference
Latent Tuberculosis	Rifapentine Isoniazid	III/II	Exposure of Rifapentine among participants by median area under the curve (AUC)	Centers for Disease Control and Prevention	up to 12 Years (Child)	[65, 66]
Tuberculosis	MTBVAC and BCG	I/II	Protection and reactogenicity of MTBVAC by calculating at increasing dose levels the number of participants with AEs and SAEs compared to the BCG vaccine	International AIDS Vaccine Initiative	18 to 50 (Adult)	[67, 68]
Reduce Inflammation After Tuberculosis, PulmonaryTreatment Completion	Atorvastatin 40mg	III/II	Total lung glycolysis (TLG) on PET/CT imaging	University of Cape Town	18 to 65 Years (Adult, Older Adult)	[69, 70]

Augment Treatment Effectiveness for Drug-sensitive Tuberculosis	Rifampicin Isoniazid Pyrazinamide Ethambutol Linezolid Clofazimine Rifapentine Levofloxacin Bedaquiline	III/II	Unsatisfactory clinical result, following randomization, at week 96.	University College, London	18 to 65 Years (Adult, Older Adult)	[71]
Sputum Smear-Positive Pulmonary Tuberculosis	Gatifloxacin Isoniazid Levofloxacin Linezolid Moxifloxacin	I/II	Under AUC curve: Sputum Bacillary Loads Difference in Sputum Bacillary Loads Extended Early Bactericidal Activity (EBA) From Days 2 to 7; Fluoroquinolones /Isoniazid (INH) Comparison	National Institute of Allergy and Infectious Diseases (NIAID)	18 to 65 Years (Adult, Older Adult)	[72, 73]
HIV-Negative Adults With & Without Latent Tuberculosis Infection	H56ug/IC31n mol	I/IIa	Number and percentage of adverse effects reported and unsolicited Reported Post Day 0 Vaccination.	Aeras	18 Years to 50 Years (Adult)	[74, 75]
Early Bactericidal Activity of Pulmonary Tuberculosis	TBA-7371 and HRZE	II	Slope of Average Change per Day, From Day 0 to Day 14	Bill & Melinda Gates Medical Research Institute	18 to 60 Years (Adult)	[76, 77]
Multidrug-Resistant Tuberculosis (MDR-TB) in HIV-Infected and HIV-Uninfected Children With MDR-TB	Delamanid (Tuberculosis) Optimized multidrug background regimen (OBR) for children with MDR-TB (HIV Infection)	I/II	Rate of Adverse Events Grade 3 or 4 (AEs) The frequency of Grade 3 or Grade 4 AEs measured by the Clinical Management Committee (CMC) in relation to DLM Frequency of deaths of participants	National Institute of Allergy and Infectious Diseases (NIAID)	up to 18 Years (Child, Adult)	[78]
Infants, adolescents and teenagers with Multidrug-Resistant Tuberculosis HIV-Infected and HIV-Uninfected	Bedaquiline	I/II	Rate of initiation of therapy by participants because of a drug-related adverse effect Total QTc period equivalent to or greater than 500 msec Frequency of unstable dysrhythmias that require care and hospitalisation Incidence of mortality	National Institute of Allergy and Infectious Diseases (NIAID)	up to 18 Years (Child, Adult)	[79]
Tuberculosis	Recombinant Mycobacterium Tuberculosis Vaccine Freeze-dried (AEC/BCO2)	Ib	The proportion of adverse reactions following intramuscular injection	Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd.	18 to 45 Years (Adult)	[80]

Several novel or repurposed antimicrobial drugs for MDR tuberculosis are in advanced trials, and two more antimicrobial drug candidates are in early-stage trials. A number of studies are being conducted to reduce the length of treatment for MDR and drug-susceptible tuberculosis. A wide variety of candidate host-directed therapies is being developed to accelerate infection eradication, prevent new drug resistance, and avoid long-term lung damage [64] Many global initiatives now have unique ways to combat the spread of tuberculosis,

enabling funders to combine several national and international programmes for the greatest overall effect, thanks to strategic partnerships for the preparation and testing of clinical trials between high-income and middle-income countries and low-income countries.

5. CONCLUSION

Anti-TB medicines that are currently in use were produced 40 years ago, and a new generation of TB drugs is urgently needed to address the problems of drug-resistant tuberculosis and mycobacterium persistence. Current TB epidemiology study suggests that focused awareness-raising programmes are also needed to raise clinician awareness of the disease's severity. The first step in the fight against tuberculosis, early detection and treatment are critical for achieving the best results and reducing the risk of infection to others. Special consideration should also be given to so-called high-risk communities more likely to become infected with tuberculosis and to acquire active diseases in order to maintain a vicious group transmission cycle. Care services are the essential to monitoring and prevention methods that are ultimately focused on the accurate diagnosis and treatment of latent TB patients and persons.

Conflict of interest

The authors declare no conflict of interest.

6. REFERENCES

1. FriedmanEE , Dean HD, Duffus WA, et al. *Public Health Rep.*, 2018;**133(4)**:392-412.
2. Pai M, Minion J, Sohn H, Zwerling A, Perkins MD, et al. *Clin. Chest Med.*, 2009; **30(4)**:701-716.
3. Pai M, Ramsay A, O'brien R,et al. *PLoS Med.*, 2008 22; **5(7)**:e156.
4. Storla DG, Yimer S, Bjune GA, et al. *BMC public health*, 2008;**8(1)**:1-9.
5. Richeldi L. *Am. J. Respir. Crit. Care Med.*, 2006; **174(7)**:736-742.
6. Nahid P, Pai M, Hopewell PC, et al. *Proc Am Thorac Soc.*, 2006; **3(1)**:103-110.
7. Er O, Temurtas F, Tanrıkulu AÇ, et al. *J. Med. Syst.*, 2010; **34(3)**:299-302.
8. Anna KP, April CP, Timothy RS, et al. *Curr Respir Care Rep.*, 2013; **2(4)**:199-207.
9. Hirsh AE, Tsolaki AG, DeRiemer K, et al. *Proc Natl Acad Sci USA.,* 2004;**101**:4871-4876.
10. Rothschild BM, Martin LD, Bercovier HL, et al. *Clin Infec Dis.*, 2001; **33**:305-311.
11. James EF, Frederick LR, Michael Bloh A, et al. *Clinical Infectious Diseases*, 2002; **34(1)**:e12-e13.
12. Markowitz, Norman. *Ann Intern Med.*, 1993; **119(3)**:185-193.
13. Garg SK, Tiwari RP, Tiwari D, Singh R, Malhotra D, Ramnani VK, et al. *J. Clin. Lab. Anal.*, 2003; **17(5)**:155-163.
14. Sima Halevy, Arnon D, Cohen, et.al. *J Am Acad Dermatol.,* 2005; **52**:254-261.
15. Samantha Ribeiro, Kelly Dooley, Judith Hackman, et al. *BMC Infect. Dis.,* 2009; **9**:23.
16. Mazurek GH, Jereb J, Lobue P, et al. *MMWR Recomm Rep.,* 2005; **54(RR-15)**:49-55.
17. Sharma SK, Mohan A, Sharma A, et al. *The Indian Journal of Medical Research*, 2012; **135(5)**:703.
18. Brock I, Munk M E, Kok-Jensen A, Andersen P, et al. *International Journal of Tuberculosis and Lung Disease*, 2001; **5(5):**462-467.
19. Kumar Vinay, Abbas Abul K, Fausto, et al. Robbins Basic Pathology (8th ed.). *Saunders Elsevier.*, 2007; 516-522.
20. Davis J Lucian. *Lancet Infect Dis.,* 2013; **13(2)**:147-154.
21. Lighter J, Rigaud M, Eduardo R, Peng CH, Pollack H, et al. *Pediatrics*, 2009; **123(1)**:30-37.
22. Turchiano, Nguyen, Fierman, Lifshitz, et al. *Int. J. Environ. Res. Public Health*, 2013; 1-4.
23. Siddiqi Kamran, et al. *The Lancet Infect. Dis.* 2003; **3**:288.
24. Raqib JR, Kamaluddin AK, et al. *J. Infect. Dis.* 2003; **188**:364-370.
25. Kothadia SN, Deshmukh S, Saoji AM, et al. *Indian J Pathol Microbiol.,*1990; **33**:68-73.
26. Al-Hajjaj, Gad-el-Rab MO, Al-Orainey Io, et al. *Tuber Lung Dis.*, 1999; **79(3)**:181-185.
27. Yilmaz A, Ece F, Bayramgurler B, et al. *Respir Med.*, 2001; **95**:666-669.
28. Huebner RE, Schein MF, Bass JB Jr, et al. *Clin Infect Dis.*, 1993; **17**:968-975.
29. Donald PR. *Curr. Opin. Pulm. Med.*, 2000; **6**:187-192.
30. Dhand R, Ganguly NK, Vaishnavi C, et al. *J Med Microbiol.*, 1988; **26**:241-243.
31. Wang CR, Liu MF, Chen MY, et al. *Lancet,* 1989; **22(2)**:97-104.
32. Lu CZ, Qiao J, Shen T, et al. *Lancet,*1990; **336**:10-13.
33. Chang HS, Sack DA, et al. *Clin Diagn Lab Immunol.*, 2001; **8**: 482-488.

34. Raqib, Rahman J, Kamaluddin AK, et al. *J Infect Dis.*, 2003; **188(3)**:364-370.
35. Cohen RA, Muzaffar S, Schwartz D, et al. *Am J Respir Crit Care Med.*, 1998; **157**:156-161.
36. Van Pinxteren LA, Ravn P, Agger EM, Pollock J, Andersen P, et al. *Clin Diagn Lab Immunol.*, 2000; **7(2)**:155-160.
37. Ridderhoff J, Williams L, Legois S, Bussen, et al. *J Clin Microbiol.,* 2003; **41(11)**:5258-5261.
38. Starke JR. *Tuberculosis*, 2003; **83**:208-212.
39. Zar HJ, Hanslo D, Apolles P, et al. *Lancet*, 2005; **365**:130-134.
40. Marais BJ, Gie RP, Hesseling AC, et al. *Pediatrics*, 2006; **118**: e1350-1359.
41. Morris CDW. *Thorax*, 1990; **45**:912-913.
42. Schluger NW. *Clin. Chest Med.*, 2007; **28**: 773-781.
43. Perez-Guzman C, Vargas MH, Torres-Cruz A, et al. *Chest*, 1999; **116**:961-967.
44. Knechel NA. *Crit Care Nurse.*, 2009; **29(2)**:34-43.
45. Sreeramareddy CT, Panduru KV, Menten J, Van den Ende J, et al. *BMC Med.,* 2009; **9(1)**:1-0.
46. Christoph Lange, Toru Mori, et al. *Respirology,* 2010; **15**:220-240.
47. Breen RA, Hardy GA, Perrin FM, et al. *PLoS ONE*, 2007; **2**:e1335.
48. Nemeth J, Winkler HM, Zwick RH, et al. *J. Intern. Med.,* 2009; **265**:163-168.
49. Barry SM, Lipman MC, Bannister B, et al. *J. Infect. Dis.*, 2003; **187**:243-250.
50. Breen RA, Barry SM, Smith CJ, et al. *Thorax*, 2008; **63**:67-71.
51. Brightling CE. *Chest*, 2006; **129**:1344-1348.
52. Villegas MV, Labrada LA, Saravia NG, et al. *Chest*, 2000; **118**:1355-1364.
53. Sherman LF, Fujiwara PI, Cook SV, Bazerman LB, Frieden TR, et al. *International Journal of Tuberculosis and Lung Disease*, 1999;**3(12)**:1088-1095.
54. Nagesh BS, Sehgal S, Jindal SK, et al. *Chest,* 2001; **119**:1737-1741.
55. Lima DM, Colares JK, da Fonseca BA, et al. *Chest*, 2003; **124**: 909-914.
56. Kim SY, Park YJ, Kang SJ, et al. *Diagn. Microbiol. Infect. Dis.,* 2004; **49**:13-18.
57. Zemlin AE, Burgess LJ, Carstens ME, et al. *Int. J. Tuberc. Lung Dis.,* 2009; **13**:214-220.
58. Baba K, Hoosen AA, Langeland N, et al. *PLoS ONE,* 2008; **3**:e2788.
59. Liang QL, Shi HZ, Wang K, et al. *Respir. Med.,* 2008; **102**: 744-754.
60. Greco S, Girardi E, Masciangelo R, et al. *Int. J. Tuberc. Lung Dis.*, 2003; **7**:777-786.
61. Jiang J, Shi HZ, Liang QL, et al. *Chest*, 2007; **131**:1133-1141.
62. Losi M, Bossink A, Codecasa L, et al. *Eur. Respir. J.,* 2007; **30**:1173-1179.
63. Lange C, Hellmich B, Ernst M, et al. *Nat. Clin. Pract. Rheumatol.,* 2007; **3**:528-534.
64. Kobashi Y, Shimizu H, Mouri K, et al. *Scand. J. Infect. Dis.,* 2009; **41**:232-236.
65. Lee LN, Chou CH, Wang JY, et al.*Clin. Microbiol. Infect.,* 2009; **15**:173-179.
66. Hooper CE, Lee YC, Maskell NA, et al. *Curr. Opin. PulmMed.*, 2009; **15**:358-365.
67. Hasaneen NA, Zaki ME, Shalaby HM, et al. *Chest*, 2003; **124**:2105-2111.
68. Gopi A, Madhavan SM, Sharma SK, et al. *Chest*, 2007; **131**:880-889.
69. Thwaites G, Fisher M, Hemingway C, Scott G, Solomon T, Innes J, et al. *J. Infect.*, 2009; **59(3):**167-187.
70. Dunn R, ZondaghI, et al. *S. Afr. Med. J.,* 2008; **98**:360-362.
71. Jung NY, Jee WH, Ha KY, et al. *Am. J. Roentgenol.,* 2004; **182**:1405-1410.
72. Fujita J, Bandoh S, Kubo A, et al. *Int. J. Tuberc. Lung Dis.,* 2006; **10**:222-226.
73. Pipavath SNJ, Sharma SK, Sinha S, et al. *Indian J. Med Res.,* 2007; **126**:193-198.
74. Sawy MS, Jayakrishnan B, Behbehani N, et al. *Saudi. Med. J.*, 2004; **25**:1459-1463.
75. Scarborough M, Thwaites GE, et al. *Lancet Neurol.,* 2008; **7**:637-648.
76. Janse van Rensburg P, Andronikou S, van Toorn R,et al.*Pediatr. Radiol.,* 2008; **38**:1306-1313.
77. Que Y, Wang X, Liu Y, et al. *Eur. J. Radiol.*, 2009; **70**:331-335.
78. Krishnan P, Vayoth SO, Dhar P, et al. *ANZ J. Surg.*, 2008; **78**:987-989.
79. Riquelme A, Calvo M, Salech F, et al. *J. Clin. Gastroenterol.,* 2006; **40**:705-710.
80. Biglino A, Crivelli P, Concialdi E, et al. *Infection.*, 2008; **36**:601-604.